INSTITUTE REPORT NO. 448

Vitamin A Supplementation Effects on Photopic and Scotopic Visual Function and Measures of Vitamin A Status

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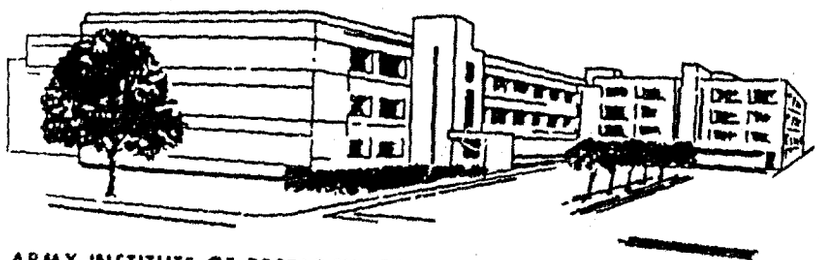
Division of Ocular Hazards Research

January 1990



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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report 448	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Division of Ocular Hazards 6b. OFFICE SYMBOL (If applicable) SGRD-ULY-OH	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco,CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M161102 BS15; TASK NO. CF; WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Vitamin A Supplementation Effects on Photopic and Scotopic Visual Function and Measures of Vitamin A Status

12. PERSONAL AUTHOR(S)
Harry Zwick, Betty Burri and Edwin S. Beatrice

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM 1986 TO 1989	1989, November	13

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	night vision, vitamin A, Retinol Binding Protein, dark adaptation, Vitamin A deficiency, contrast sensitivity, supplementation

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

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20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
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22a. NAME OF RESPONSIBLE INDIVIDUAL Donald G. Corby, COL, MC, Commanding	22b TELEPHONE (Include Area Code) (415)561-3600
	22c. OFFICE SYMBOL SGRD-ULZ

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

Abstract

We investigated the effects of vitamin A supplementation in an individual with abnormally low vitamin A-containing transthyretin-bound retinol binding protein (RBP). Measures of spectral dark adaptation and spatial contrast sensitivity suggest a differential return of parafoveal and foveal receptor systems during supplementation. Parafoveal cone systems appear to return more rapidly than foveal cones and rods. Post-supplementation measurements of spectral dark adaptation demonstrated a crossing of spectral dark adaptation functions at 6.5 minutes, close to the appearance of the rod cone break attributed to achromatic measurements of dark adaptation. Contrast sensitivity for the finest spatial frequency showed a delay in its return to near normal levels relative to recovery of mid to low spatial frequencies. While measures of serum retinol, total RBP, and free RBP increased during supplementation, transthyretin-bound RBP failed to show any increase relative to its abnormally low pre-supplementation level. Bodily stores of vitamin A either require more time to return to normal levels than retinol delivered to the retina or this individual possessed a specific genetic deficiency in the bodily mechanism responsible for storing vitamin A.

Vitamin A Supplementation Effects on Photopic and Scotopic Visual Function and Measures of Vitamin A Status

INTRODUCTION

We have investigated in an otherwise healthy adult male, who had markedly elevated dark adaptation, clinically normal serum retinol, and a pale appearing retina. We measured the effects of vitamin A supplementation on spectral dark adaptation and contrast sensitivity. Three biochemical tests of vitamin A status were utilized: 1) serum retinol; 2) total immunologically active retinol binding protein (RBP) as determined by radial immunodiffusion; and 3) vitamin A-containing free- and transthyretin-bound RBP as determined by high performance liquid chromatography (HPLC).

Spectral measures of dark adaptation utilizing red and green light emitting diodes (LEDs) were used to provide greater separation of rod and cone function during supplementation (1-3). Spatial vision was assessed by contrast sensitivity, so that fine as well as gross spatial mechanisms could be examined during supplementation. Retinal receptor thinning induced by vitamin A deprivation (4) may alter foveal receptor matrix and cause changes in visual acuity and spatial function similar to functional and morphological alterations observed in photic maculopathy (5, 6).

Serum retinol is one of the most commonly used methods for detecting vitamin A deficiency, but it is influenced by protein-calorie malnutrition or alcoholism and is poorly correlated to vitamin A body stores except in cases of true deficiency or toxicity (7). Retinol-binding protein is the major retinol-transporting protein in blood serum (8). Usually RBP circulates in blood in a 1:1 complex with transthyretin (8), but a significant fraction of free RBP (not complexed to transthyretin) is believed to be present in the blood. Total RBP concentration, as measured by radial immunodiffusion, is influenced by the amount of vitamin A stored in the liver and can be used to detect vitamin A deficiencies (9, 10). Unfortunately, total RBP is also influenced by liver disease and protein-calorie malnutrition (11, 12) and is not highly correlated to vitamin A body stores except in deficiency.

Recent investigations have developed techniques for measuring free and transthyretin-bound RBP (13, 14). Measures of free RBP correlate highly to serum retinol and total RBP in humans with adequate or marginal vitamin A status. On the other hand, transthyretin-bound RBP is more highly correlated to liver (and presumably, total body) vitamin A stores measured in marginally deficient or subtoxic rats than free RBP, total RBP, or serum retinol (15). Measures of transthyretin bound RBP were employed to compare normal and human retinal disease (16).

Methods

Figure 1 presents a schema of the apparatus and procedures used to measure spectral dark adaptation. Spectral test stimuli were comprised of red and green LED sources. The spectral output of these sources are shown in the lower left insert of this Figure as the C and E emission spectra. Other emission spectra shown were not employed in the present study. Pulse width modulation of LEDs sources was used to control the apparent intensity of each LED source. Visual threshold LED pulses from 1 microsecond to 1 millisecond depend upon energy. LED amplitude and pulse are reciprocal in determining visual threshold (1-3).

Red and green LED sources were arrayed in individual crosses with a central fixation diode (upper left insert). Measurements of dark adaptation were made at either 16 or 2 degrees from fixation using four LEDs equidistance from the fixation. Red and green crosses were alternated during threshold measurements. The subject's task was to fixate the central LED, to hold his fixation on the central LED until some other source of light appeared in his peripheral view. At this time, he depressed his response button until the light in his peripheral view disappeared while maintaining central fixation. LED threshold measurements were alternately measured in this manner through the course of dark adaptation.

Dark adaptation functions were obtained following 2 minute light adaptation in a 36-inch hemisphere, fitted with chin rest and head support and indirectly illuminated with a tungsten source that produced a uniform illumination of 100 cda/m^2. In the upper right, a sample dark adaptation function from one individual is presented relative to upper and lower bounds representing two standard deviations above and below the norm for this test (1-

3). The dynamic range of this function and the absolute threshold measured with the green LED are equivalent to that obtained by Sloan (17).

Measurement of contrast sensitivity was made with a video grating contrast sensitivity apparatus (Nicolet) over a spatial frequency range from 0.5 to 22.8 Hz/degree.

Serum retinol was determined by a Series 400 liquid chromatograph equipped with an LC 90 UV-Vis spectrophotometric detector and an LC 100 computing integrator (Perkin-Elmer Corp). Chromatography was on a C18 reverse phase column (18, 19). Total immunologically active retinol-binding protein (RBP) was determined by radial immunodiffusion (Behring Diagnostics) according to manufacturer's procedure.

Vitamin A-containing free- and transthyretin-bound RBP were measured by molecular exclusion HPLC with fluorometric detection as previously described with the exception that Acro LC 13 filters were used instead of Millex filters (14).

Results

In Figures 2a and 2b, spectral dark adaptation functions measured for the red and green LEDs show dramatic changes in the shape of the dark adaptation prior to, during, and post-supplementation periods. The functions for the red LED appeared to return to near normal levels within the first 3 days of supplementation. The function for the green LED shows a similar trend over the 6 or 7 minutes of dark adaptation, with an additional increase in sensitivity that appeared near the end of supplementation and increased to the values shown in the 13-day post-supplementation function.

Prior to supplementation, spectral dark adaptation functions failed to cross. Following supplementation, a crossover occurs between 6 and 7 minutes of dark adaptation (Figure 3).

In Figure 4, log sensitivity is plotted as a function of days prior to, during, and post-supplementation. Changes in sensitivity or contrast sensitivity show a steady improvement during and after supplementation with exception of the 23 Hz/deg grating, which shows a dramatic increase in sensitivity just prior to the termination of

the supplementation period. While full recovery does not occur, sensitivity for all spatial frequencies are within or very close to one standard deviation of the normative sensitivity values for this test of contrast sensitivity.

Vitamin A supplementation increased all biochemical measurements of vitamin A (Figure 5) measured with the exception of transthyretin-bound retinol binding protein which failed to change throughout the supplementation and post-supplementation measurement period.

Clinical measures of visual acuity increased from approximately 20/30 to 20/20 about the same time that contrast sensitivity for the fine spatial frequency, 23 Hz/deg, occurred. An apparent darkening of the macular was observed during the supplementation interval.

Discussion

The sensitivity of vitamin A-containing transthyretin-bound RBP as an indicator of body levels of retinol is demonstrated in this case. Vitamin A supplementation dramatically restored photopic and scotopic measures of visual function. The relative increase in serum retinol, total immunological RBP, and vitamin A-containing free RBP during supplementation demonstrates the ability of these measures to reflect retinol concentrations at the retina.

Transthyretin RBP may not have increased during this time because vitamin A body stores require more time to normalize relative to free RBP. On the other hand, the longer time for restoration of transthyretin-bound RBP may result from either an acquired or genetic deficiency or defect in the storage mechanism of retinol or the RBP complex.

Measurements of spectral dark adaptation during supplementation indicate that cone function returns more rapidly than rod function, as indicated by comparing peripheral measures of spectral dark adaptation for the red and green LED functions (Figures 2 and 3). A definite crossover in spectral dark adaptation does not occur until after supplementation. At this time a crossover occurs between 6 and 7 minutes, which is comparable to the rod cone break measured in achromatic dark adaptation functions. Measurement of contrast sensitivity further suggests that the return of the fine spatial frequency mechanism, which mediates visual acuity and should reflect

foveal cone function, is initially delayed relative to the return of sensitivity of mid to low spatial frequencies.

These differential effects in the recovery of visual sensitivity may reflect morphological differences between parafoveal and foveal cones. The thinner and longer foveal cone outer segments may have more difficulty in absorbing the vitamin A molecule, especially with the tightness of receptor packing in the normal human fovea, requiring more time for restoration of normal retinal kinetic functions. If vitamin A deficiency does produce a thinning of human retinal photoreceptors, as shown by Herron and Riegel (4) for the rat rod receptors, then it is possible that such effects may alter the foveal photoreceptor matrix in the human eye, consequently resulting in a small acuity change associated with vitamin A deficiency. Changes in foveal packing following photic macula damage has been hypothesized to account for recovery in studies of photic foveal damage (5, 6). Vitamin A supplementation may gradually restore the altered foveal receptor matrix, accounting for the delayed increase in fine spatial frequency sensitivity.

We suggest that these structural factors as well as differential kinetics of rod and cone vision account for the hierarchical return of central and peripheral retinal receptor function exhibited by this patient during vitamin A supplementation.

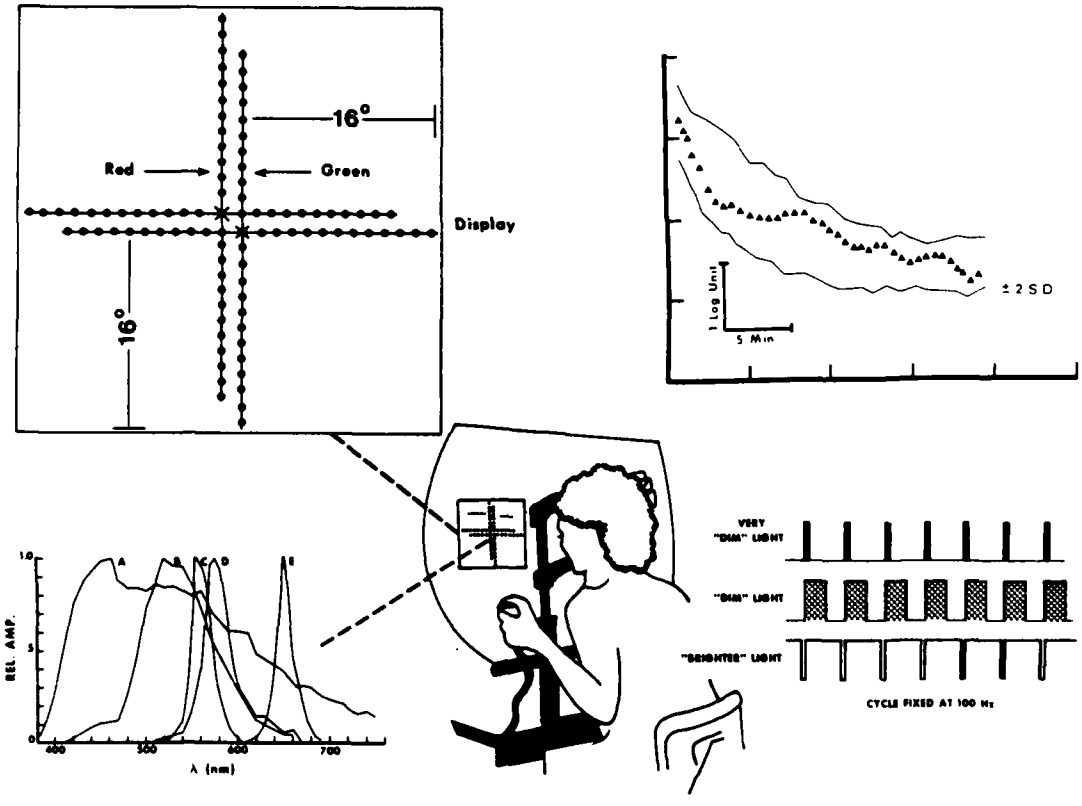

Figure 1. Schematic illustration of LED dark adaptation. Upper right: sample dark-adaptation function showing how the threshold pulse width decreases in dark adaptation. Lower right shows the correlation of pulse width with apparent intensity. The relative spectral transmission curves of the LED sources available to this apparatus are shown in the lower left corner. The C and E diodes were employed in this experiment. The LED light sources were arranged in a cross pattern (upper right). They were equated in peak luminance at approximately (12 X 10^{-6} lm/(cm^2 sr) by using radiometric measurement from an EG&G 580 Radiometer. All LEDs in both horizontal and vertical meridians were adjusted to meet this luminance output.

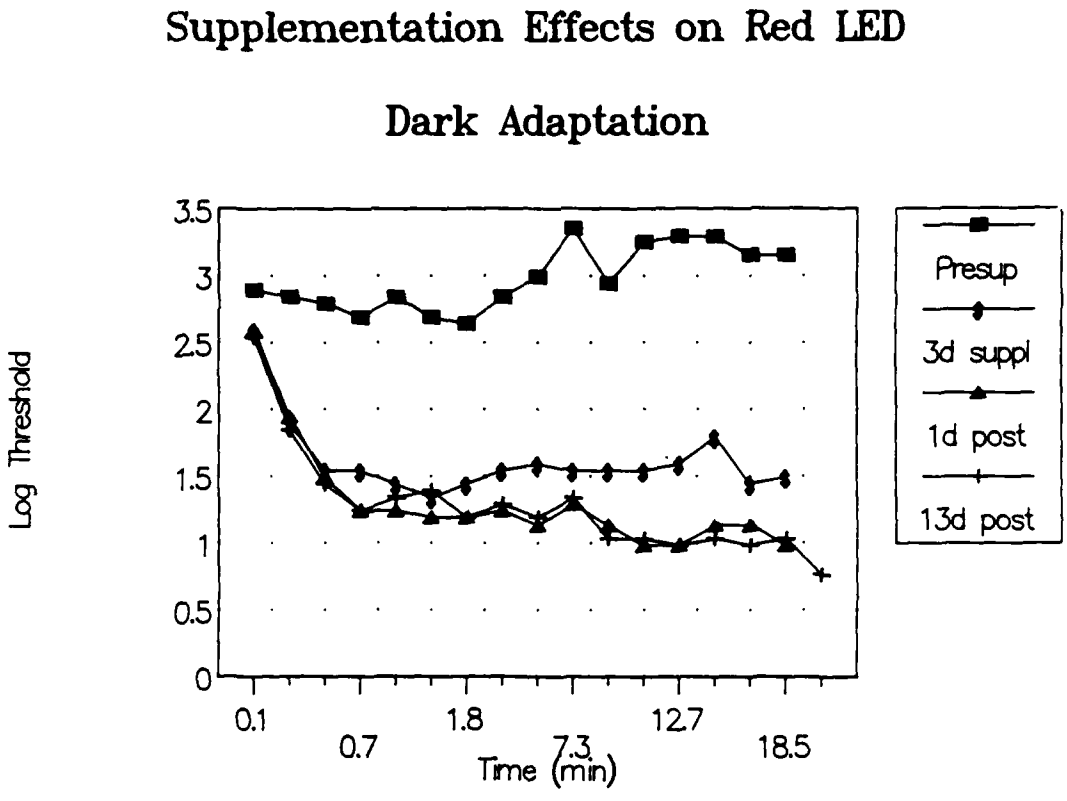

Figure 2a. Spectral dark adaptation for the red LED measured prior to, during, and postvitamin A supplementation.

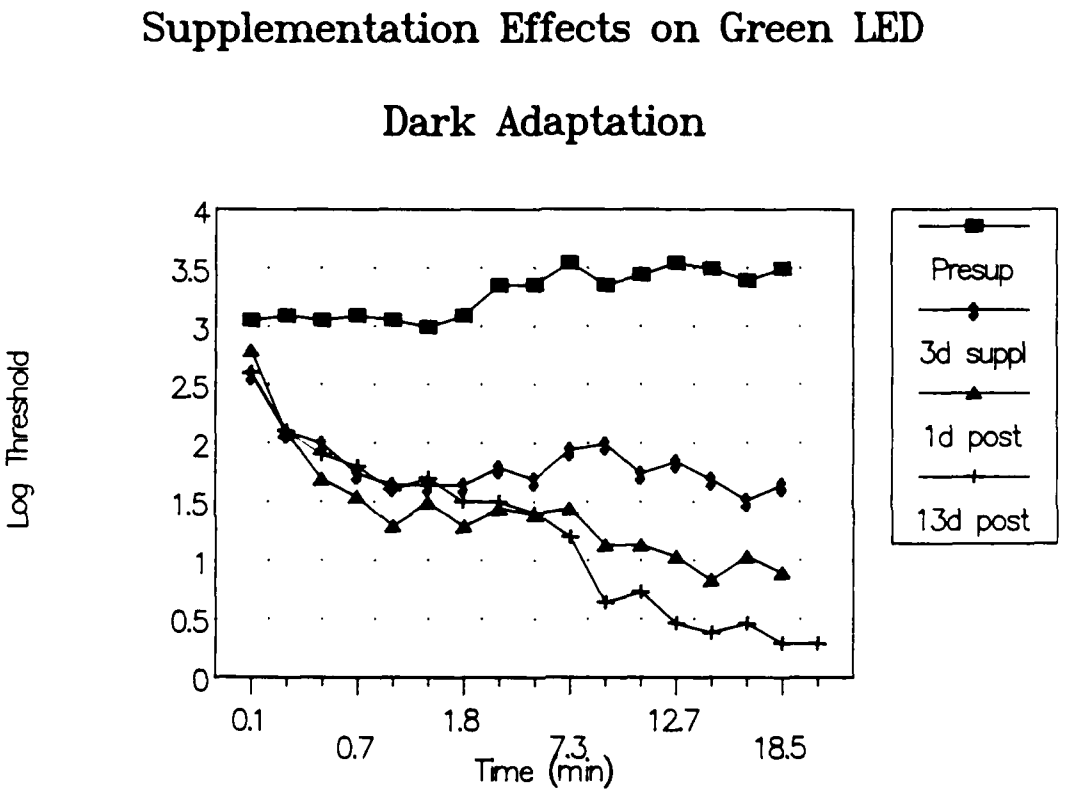

Figure 2b. Spectral dark adaptation for the green LED measured prior to, during, and postvitamin A supplementation.

Comparison of Spectral Dark Adaptation Following Vitamin A Supplementation

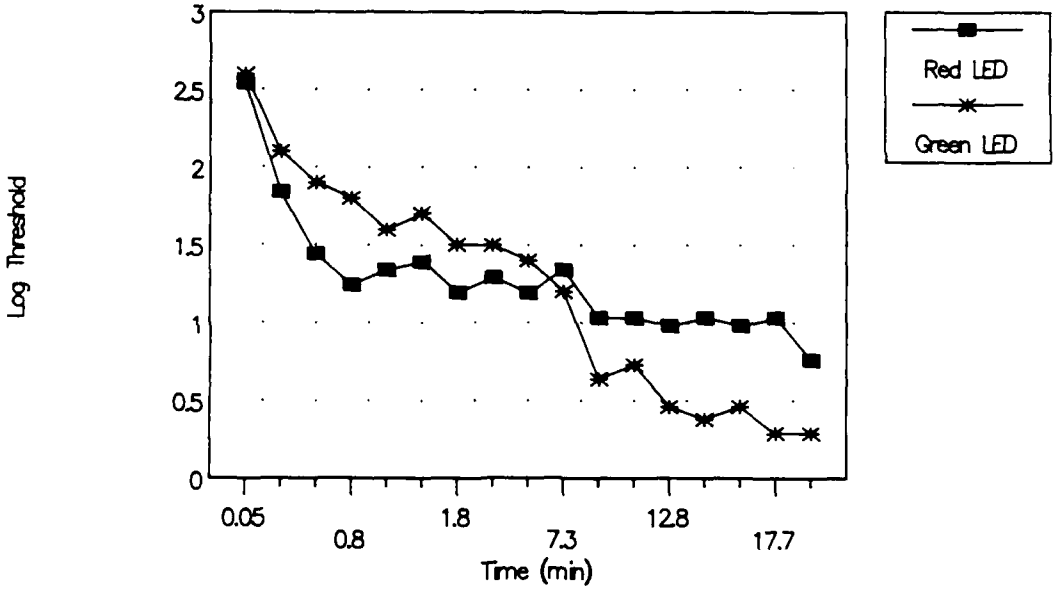

Figure 3. Postsupplementation red and green dark adaptation functions showing crossover between 6 and 7 minutes.

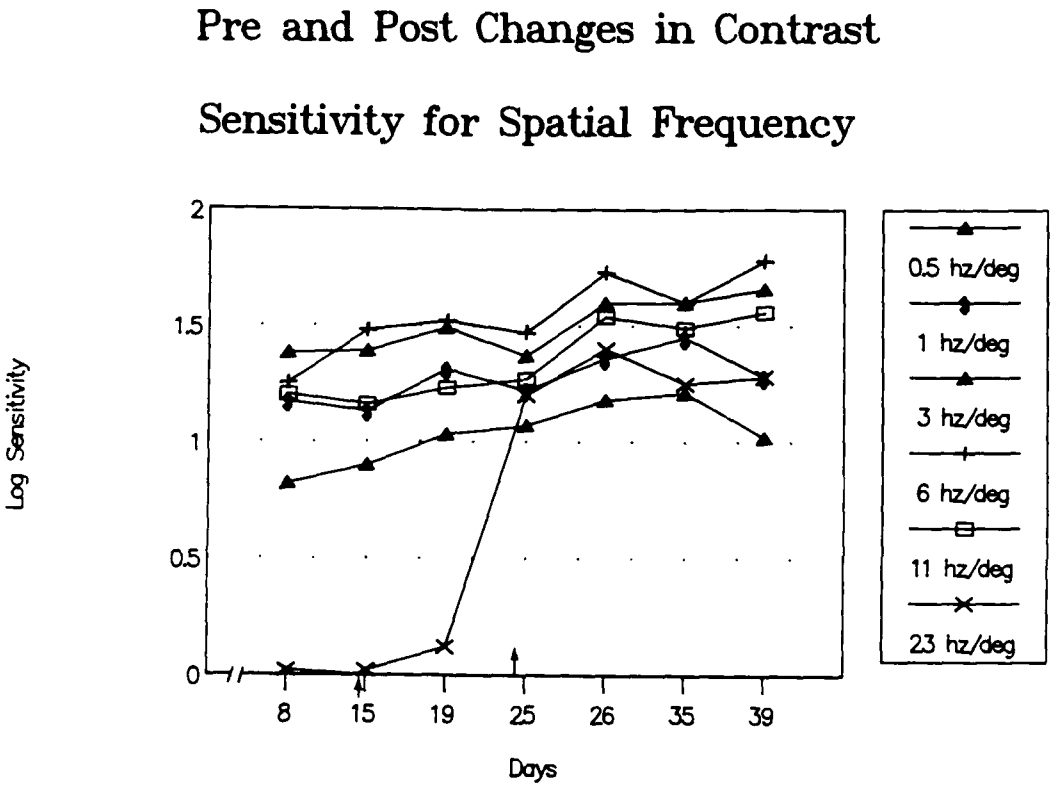

Figure 4. Log contrast sensitivity vs. days prior to, during, and postvitamin A supplementation for different spatial frequencies. Vertical arrows on the abscissa represent the beginning and end of the supplementation period. The points at the right represent the normal contrast sensitivity function for these spatial frequencies.

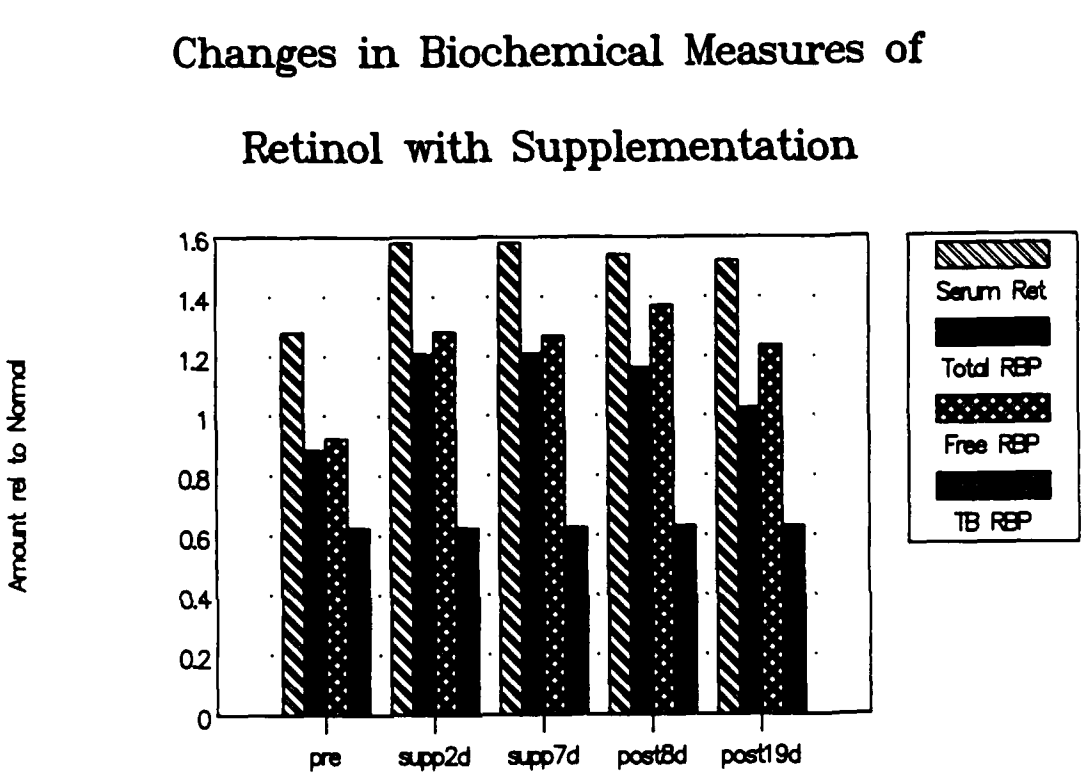

Figure 5. Relative changes in biochemical measures of retinol-Serum Retinol (Serum Ret), Total RBP, Free RBP, and Transthyretin bound retinol binding protein (TB RBP) are shown prior to, during, and postvitamin A supplementation.

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